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Isolation and identification of insecticidal compounds from *Tephrosia purpurea* (Fabaceae) bark and their insecticidal activity

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Abstract: In order to determine insecticidal compounds from the methanol extracts of *Tephrosia purpurea* bark, the active compounds were isolated by activity-guided fractionation with column chromatography and identified based on NMR (nuclear magnetic resonance) and MS (mass spectrometry) data. Slide-dip method was performed to determine the insecticidal activities of each compound against *Myzus persicae* adults, and topical application was conducted to determine contact toxicity of each compound against the 3rd instar larvae of *Pluttella xylostella*. Ten known compounds were isolated and identified, *i. e.*, 12a-hydroxyrotenone, 4′-hydroxyemoroidocarpan, pachyrrhizine, rotenone, 6-methoxycoumarin, (-)-edunol, obovatin, pongachin, 12-acetyelliptinol and 2-hydroxyrotenone. All these compounds exhibited insecticidal activity against the 4th instar larvae of *Aedes albopictus* with the LC_{50} value being 12.5, 22.1, 25.0, 34.1, 43.4, 58.4, 121.9, 191.0, 219.8 and 250.0 mg/L, respectively at 24 h after treatment. Moreover, three compounds (4′-hydroxyemoroidocarpan, rotenone and 12a-hydroxyrotenone) exhibited insecticidal activity against *M. persicae* adults and the 3rd instar larvae of *P. xylostella* with their corresponding LC_{50} values being 49.9, 1.9 and 0.9 mg/L against *M. persicae* adults, and with the LD_{50} values being 49.8, 197.1 and 40.9 μg/individual against *P. xylostella* larvae, respectively. Eight known compounds, *i. e.*, 4′-hydroxyemoroidocarpan, 2-hydroxyrotenone, 6-methoxycoumarin, pachyrrhizine, (-)-edunol, 12-acetyelliptinol, pongachin and obovatin, were isolated from *T. purpurea* bark for the first time. The elucidation of the structure of these phytochemicals and their insecticidal activity is important not only for understanding the insect-plant relationships, but also for assessing the potential of this plant as botanical insecticide to be explored and utilized.

Key words: *Tephrosia purpurea*; insecticidal compounds; insecticidal activities; *Aedes albopictus*; *Pluttella xylostella*; *Myzus persicae*

1 INTRODUCTION

At present, the control of insect pests is primarily dependent on chemical insecticides such as organophosphorus compounds and the synthetic pyrethroids. However, the long-term use of synthetic insecticides has caused environmental contamination, toxicity to non-target organisms and resurgence of target pests (Isman, 2000). These problems promote researchers to develop new environmentally friendly pesticides.

As is well known, plant secondary metabolites play an important role in protection of the plants from being damaged by pests, germs and adverse climate (Chen, 2004). In fact, some phytochemicals have been used to control pests for centuries (Huang *et al.*, 2010). Even today, some farmers still use the dried stems of *Nicotiana tabacum* and dried flowers of *Rhododendron molle* to control pests in China (Huang *et al.*, 2010). Botanicals with insecticidal activities are the potential sources to be utilized as insecticides (Xu, 2001). Therefore, most researches on pesticides focused on seeking and

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exploring new types of botanical pesticides, which are biodegradable into nontoxic products and suitable for the control of insect pests in the integrated management programs (Ben *et al.*, 2000; Nathan *et al.*, 2007; Pavela and Herda, 2007).

Tephrosia purpurea (Fabaceae) is a tropical and subtropical species. Previous studies revealed that some flavonoids occurred in this plant and focused on medical values of these compounds and the extracts from this plant (Sinha *et al.*, 1982; Rao and Raju, 1984; Khan *et al.*, 2001), while little was reported on the insecticidal activity in the extracts from this plant. In fact, some published documents reported that its methanol extracts possessed insecticidal activities against various species of insect pests (Li *et al.*, 2007, 2011), suggesting that exploration and utilization of the plant as botanical insecticides deserve to be evaluated. It was also reported that some flavonoids in this plant are prenylated flavones (Sinha *et al.*, 1982; Rao and Raju, 1984), which undergo further substitution and cyclization leading to complex molecules (Sinha *et al.*, 1982). According to these results, more insecticidal compounds with complex chemical structure presumably occur in this plant. So the aim of this study was to isolate insecticidal compounds from *T. purpurea* bark and to determine their insecticidal activity, which may be useful for further exploration and utilization of the plant as botanical insecticides.

2 MATERIALS AND METHODS

2.1 Plant materials

The stem bark of *T. purpurea* was collected in South China Agricultural University, Guangdong province, southern China, in April 2006, and identified by Professor Zhong Ye-Cong from Guangxi Academy of Forestry. An authenticated voucher specimen (No. 200607) of this plant was deposited at College of Bio-Safety Science and Technology, Hunan Agricultural University.

2.2 Insects

Aedes albopictus larvae were reared successively in the laboratory with ten percentage yeast suspension as the food source following the method of Huang *et al.* (2010). *Pluttella xylostella* and *Myzus persicae* colonies, which were collected from container-grown cauliflowers in glasshouses, were reared respectively on cauliflowers in a thermostatic chamber, Hunan Agricultural University. All the tested insects were maintained at 25 ± 1℃, 75% – 85% RH with a 12-hour photoperiod.

2.3 Bioassays

Activity-guided method was performed to determine the insecticidal activity in the plant extracts or fractions (Li and Xu, 2007; Huang *et al.*, 2010). A volume of 24.5 mL distilled water was mixed into 500 μL dimethyl sulfoxide (DMSO) solution containing extracts or fraction in a cup, with gentle shaking to ensure a homogeneous test solution, and then 30 4th instar mosquito larvae were transferred to the cup. The control was exposed to the mixture of 24.5 mL distilled water and 500 μL DMSO. The concentration of extracts or fractions was 500 mg/L. Mortality was recorded at 24 h after treatment. For each treatment three replicates were carried out.

Each compound was dissolved with acetone, and then diluted into a series of five different concentrations. Insecticidal activity in each compound against mosquito larvae was determined by the above-mentioned activity-guided method. Topical application (Huang, 2000) was conducted to determine the contact toxicity of the compounds against the 3rd instar larvae of *P. xylostella*, and every time 0.1 μL the mixture of compound and acetone was dipped on the pronotum of each larva. Slide-dip method (Huang, 2000) was performed to determine the insecticidal activity of each compound against *M. persicae* adults. Three replicates with a total of 90 insects were carried out simultaneously for each dilution. Controls were exposed to the solvent acetone alone. Mortality rate was recorded at 24 h after treatment.

2.4 Extraction and isolation of compounds from *T. purpurea* bark

The air-dried and powered bark of *T. purpurea* (2.3 kg) was placed in a stopped conical flask and continuously extracted with methanol (50 L) for 3 d at room constant temperature (28 – 30℃) with occasional stirring, and then filtered. After the extraction was done successively three times, evaporation of the combined filtrate under vacuum at 50℃ yielded a methanolic extract (257.6 g, 11.2% from the dried bark).

The methanolic extract (20.0 g per time) was suspended in a mixture (2 L) of water and methanol (4∶1, v/v) and repeatedly extracted in a 15 L glass-bottle with 10 L solvent of increasing polarity starting with petroleum ether (PE), then trichloromethane ($CHCl_3$), and finally ethyl acetate (EtOAc). Thus 257.6 g methanolic extract yielded petroleum ether- (22.3 g), $CHCl_3$- (95.0 g), EtOAc- (20.6 g), and H_2O-soluble (119.2 g) residues.

These residues were subjected to the

insecticidal assays against the 4th instar larvae of *A. albopictus* and the $CHCl_3$-soluble residue showed the most potent activity. This residue (95.0 g) was subjected to silica gel column chromatography (100 – 200 mesh) and eluted first with petroleum ether and then with a gradient of PE-EtOAc (0 – 100%) and finally MeOH, to give 147 fractions of 1 000 mL each. Verified by thin layer chromatography (TLC), fractions 5 – 16, 45 – 56, and 93 – 123 were found to be active in the insecticidal activity evaluation, and the other inactive fractions were discarded.

Fractions 5 – 16 were applied to TLC, and eluted with PE-EtOAc (90 : 10, v/v) to give compound A (56.3 mg). Fractions 45 – 46 were applied to a silica gel column (200 – 300 mesh) again, and eluted with petroleum ether-EtOAc (15:85, v/v) to give 97 subfractions. These subfractions were combined on the basis of the TLC results, and then the residues were used for the insecticidal assays against *A. albopictus* larvae. Subfractions 26 – 37, which exhibited insecticidal activity, were further subjected to a silica gel (200 – 300 mesh) column, eluted with PE-EtOAc (90: 10, v/v) to give compound H (32.7 mg) and compound J (48.7 mg).

Fractions 93 – 123 were applied to a silica gel column (200 – 300 mesh) again, and eluted with PE-EtOAc (15:85, v/v) to give 116 subfractions. These subfractions were combined on the basis of the TLC results, and then the residues were used for the insecticidal assays against *A. albopictus* larvae. Subfractions 10 – 19 and subfractions 21 – 39 exhibited insecticidal activity. Subfractions 10 – 19 were further subjected to a silica gel (200 – 300 mesh) column, and eluted with PE-EtOAc (10:90, v/v) to give compounds C (178.8 mg) and D (10.7 mg). The rest subfractions 10 – 19 were dissolved with actone, and submitted to HPLC (ODS-Symmtryprep, 7 μm, i.d. 7.8 mm × 150 mm column; MeOH-H_2O 2:1, v/v; 2 mL/min) to give compound E(94.7 mg), compound F(27.9 mg), and compound I (26.5 mg). Subfractions 21 – 39 (1.1 g) were processed again on silica gel (200 – 300 mesh) column chromatography using PE- actone (85:15, v/v) to give compound G (166.7 mg). The rest subfractions 21 – 39, which exhibited insecticidal activity, were subjected to Sephadex LH-20 column chromatography using acetone to give compound G (33.1 mg) and compound B (17.6 mg).

2.5 Identification of compounds isolated from the extracts of *T. purpurea* bark

^1H and ^{13}C-NMR were recorded by using a Bruker AVANCE-500 instrument. EI-MS and HR-EI-MS were accomplished on a Thermo Finnigan MAT-95XP instrument. TLC spots were visualized by UV irradiation (254 and 365 nm), and by spraying with the mixture of methanol and H_2SO_4 (1:1, v/v) followed by heating. Optical rotations were recorded with a Perkin-Elmer 343 polarimeter. Melting points were uncorrected and determined on an XT4A digital micromelting point apparatus.

2.6 Data statistics and analysis

The insecticidal activity tests with higher than 20% mortality in controls were discarded and then repeated. If the control mortalities ranged between 5% and 20%, they were corrected using Abbott's formula (Huang, 2000). LC_{50} (median lethal concentration) and LD_{50} (median lethal dosage) of compounds against the tested pests were calculated by Probit Analysis (DPS software, version 9.5). The 95% confidence interval, values and degrees of freedom of the χ^2 goodness of fit tests, and regression equation were calculated and analyzed. Whenever the goodness of χ^2 was found to be significant ($P < 0.05$), a heterogeneity correction factor was used in the calculation of the confidence limits.

3 RESULTS

3.1 Identification of compounds

Compound A is colourless needle crystal. ^1H NMR [500 MHz, $(CD_3)_2CO$]: δ_H 1.41 (3 H, s, $-CH_3$), 142 (3 H, s, $-CH_3$), 2.86 (1 H, dd, J = 17.1 Hz, 3.1 Hz, H-3), 3.17 (1 H, dd, J = 17.0 Hz, 12.8 Hz, H-3), 5.59 – 5.63 (2 H, m, H-2, H-3″), 5.90 (1 H, s, H-6), 6.51 (1 H, d, J = 10.0 Hz, H-4″), 7.40 (1 H, t, J = 7.4 Hz, H-4′), 7.45 (2 H, t, J = 7.7 Hz, H-2′, H-6′), 7.58 (2 H, d, J = 7.4 Hz, H-3′, H-5′), 12.22 (s, 1 H, HO-5); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ_C 29.4 ($-CH_3$), 29.7 ($-CH_3$), 44.5 (C-3), 79.9 (C-2″), 81.1 (C-2), 98.7 (C-6), 103.7 (C-10), 104.6 (C-9), 117.1 (C-4″), 128.2 (C-2′, C-6′), 128.6 (C-4′), 130.5 (C-3″), 130.6 (C-3′, C-5′), 140.9 (C-1′), 158.9 (C-8), 163.8 (C-7), 165.4 (C-5), 198.1 (C-4). The spectroscopic data is consistent with that listed in the literature (Waterman and Mahmoud, 1985; Andrei *et al.*, 2000), socompound A is identified as obovatin.

Compound B is colourless needle crystal. ^1H NMR [500 MHz, $(CD_3)_2CO$]: δ_H 1.42 (3 H, s, $-CH_3$), 1.44 (3 H, s, $-CH_3$), 2.69 (1 H, dd, J = 16.3 Hz, 3.0 Hz, 3-H), 2.95 (1 H, dd, J = 16.3 Hz, 12.7 Hz, 3-H), 3.82 (3 H, s, $-OCH_3$),

5.53 (1 H,dd, J=12.7 Hz, 3.0 Hz, H-2), 5.57 (1 H, d, J=10.0 Hz, H-3″), 6.10 (1 H, s, H-6), 6.56 (1 H, d, J=10.0 Hz, H-10), 7.37 (1 H,t, J=7.2 Hz, H-4′), 7.44 (2 H, t, J=7.8 Hz, H-2′, H-6′), 7.56 (2 H, d, J=7.2 Hz, H-3′, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 29.4 (-CH_3), 29.6 (-CH_3), 47.3 (C-3), 57.3 (-OCH_3), 79.5 (C-2″), 80.9 (C-2), 95.6 (C-6), 104.5 (C-10), 107.6 (C-8), 117.7 (C-4″), 128.0 (C-2′, 6′), 128.4 (C-4′),130.2 (C-3″), 130.5 (C-3′, 5′), 141.5 (C-1′), 160.5 (C-9), 161.4 (C-7), 164.1 (C-5), 188.8 (C-4). The spectroscopic data is consistent with that listed in the literature (Andrei *et al.*, 2000), so compound B is identified as pongachin.

Compound C is white plate crystal. mp: 162 – 164℃, $[\alpha]_D^{25}$ = – 228° ($C_{0.024}$, benzene). MS (m/z): 394 (M^+), 192 (100), 149, 191; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.77 (3 H, s, H-3″), 2.95 (1 H, dd, J=15.7 Hz, 8.1 Hz, H-2), 3.31 (1 H, dd, J=15.6 Hz, 9.8 Hz, H-2), 3.77 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.84 (1 H, d, J=3.0 Hz, H-6), 4.17 (1H, d, J=12.1 Hz, H-12a), 4.60 (1H, dd, J=12.1 Hz, J=3.0 Hz, H-6), 4.93 (1H, s, H-2″), 5.08 (1H, s, H-11), 5.24 (1H, t, J=9.30 Hz, H-2′), 6.45 (1H, s, H-4), 6.50 (1H, d, J=8.5 Hz, H-10), 6.77 (1H, s, H-1), 7.83 (1H, d, J=8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 17.1 (C-3″), 31.3 (C-3′), 44.6 (C-12a), 55.8 (C_{22}-OMe), 56.3 (C_{23}-OMe), 66.3 (C-6), 72.2 (C-6a), 87.8 (C-2′), 100.9 (C-4), 104.8 (C-10), 110.4 (C-12b), 112.6 (C-1), 112.9 (C-11a), 113.3 (C-8), 129.9 (C-2″), 143.0 (C-1″), 143.9 (C-2), 147.4 (C-3), 149.5 (C-4a), 157.9 (C-7a), 167.3 (C-9), 188.9 (C-12). Its MS data is consistent with rotenone's (Li and Xu, 2007), and the spectroscopic data is consistent with that listed in the literature (Li and Xu, 2007), so compound A is identified as rotenone.

Compound D is colourless needle crystal. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.77 (3H, s, CH_3), 2.95 (1 H, dd, J=15.7 Hz, 8.1 Hz, H-1′), 3.31 (1 H, dd, J=15.7 Hz, 9.8 Hz, H-1′), 3.82 (3 H, s, OCH_3), 4.18 (1 H, d, J=12.0 Hz, H-6), 4.60 (1 H, dd, J=12.0 Hz, 3.0 Hz, H-6), 4.91 (1 H, t, J=3.2 Hz, H-2″), 4.93 (1 H, s, H-2″), 5.07 (1 H, s, H-6a), 5.23 (1 H, d, J=5.4 Hz, H-2′), 5.25 (1 H, s, -OH), 6.44 (1 H, s, H-4), 6.50 (1 H, d, J=8.5 Hz, H-10), 6.83 (1 H, s, H-1), 7.82 (1 H, d, J=8.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 17.1 (C-3″), 31.3 (C-3′), 44.6 (C-12a), 55.9 (-OMe), 66.3 (C-6), 72.2 (C-6a), 87.8 (C-2′), 100.1 (C-4), 104.8 (C-10), 105.9 (C-12b), 112.5 (C-1), 112.9 (C-1″), 113.1 (C-11a), 113.3 (C-8), 130.0 (C-2″), 140.2 (C-2), 143.1 (C-11), 146.7 (C-4a), 146.9 (C-3), 157.8 (C-7a), 167.3 (C-9), 188.6 (C-12). The spectroscopic data is consistent with that shown in the literature (Charalambous *et al.*, 1995), so compound D is identified as 2-hydroxyrotenone.

Compound E is brown paste. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 1.76 (3 H, s, H-3″, -CH_3), 2.94 (1 H, dd, J = 15.8 Hz, 8.2 Hz, H-3′), 3.29 (1 H, dd, J = 15.8 Hz, 9.8 Hz, H-3′), 3.73 (3H, s, -OCH_3), 3.82 (3 H, s, -OCH_3), 4.50 (1 H, d, J=11.6 Hz, H-6), 4.61 (1 H, d, J=2.4 Hz, H-6), 4.59 (1 H, s, H-6a), 4.94 (1 H, s, H-2″, =CH_2), 5.07 (1 H, s, H-2″, =CH_2), 5.24 (1 H, t, J=9.0 Hz, H-2′), 6.49 (1 H, s, H-4), 6.54 (1 H, d, J=8.6 Hz, H-10), 6.56 (1 H, s, H-1), 7.83 (1 H, d, J=8.6 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C17.1 (C-3″), 31.1 (C-3′), 55.9 (-OMe), 56.4 (-OMe), 63.9 (C-6), 67.6 (C-12a), 87.9 (C-2′), 101.1 (C-4), 105.3 (C-10), 108 (C-12b) 109.5 (C-1), 118 (C-11a), 112.7 (C-2″), 113.2 (C-8), 130.1 (C-11), 142.9 (C-1″), 144.0 (C-2), 148.4 (C-4a), 151.2 (C-3), 157.7 (C-7a), 168.0 (C-9), 191.1 (C-12). The spectroscopic data is consistent with that listed in the literature (Phrutivorapongkul *et al.*, 2002), so compound E is identified as 12a-hydroxyrotenone.

Compound F is colourless needle crystal. $[\alpha]_D^{25}$ = – 265° ($C_{0.01}$, $CDCl_3$). ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ_H1.72 (3 H, s, -CH_3), 1.73 (3 H, s, -CH_3), 3.33 (2 H, d, J = 7.3 Hz, H-1′), 3.50 – 3.59 (2 H, m, H-6), 4.21 (1 H, dd, J=10.1 Hz, 4.0 Hz, H-6a), 5.33 – 5.37 (1 H, m, H-11a), 5.46 (1 H, d, J=6.6 Hz, H-2′), 5.90 (1 H, d, J=1.0 Hz, -OCH2O-), 5.92 (1 H, d, J=1.0 Hz, -OCH_2O-), 6.37 (1 H, s, H-4), 6.40 (1 H, s, H-10), 6.87 (1 H, s, H-7), 7.15 (1 H, s, H-1), 8.47 (1 H, s, -OH); ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: δ_C 18.8 (-CH_3), 26.9 (-CH_3), 29.4 (C-1′), 42.2 (C-6a), 68.1 (C-6), 80.6 (C-11a), 95.0 (-OCH_2O-), 103.1 (C-10), 104.6 (C-4), 106.9 (C-7), 113.6 (C-1a), 120.6 (C-2), 123.9 (C-6b), 124.9 (C-2′), 133.3 (C-3′), 133.5 (C-1), 143.5 (C-4a), 149.9 (C-9), 156.4 (C-10a), 156.7 (C-4a), 158.0 (C-3). The spectroscopic data is consistent with that listed in the literature (Reyes-Chilpa *et al.*, 1994), so compound F is identified as (-)-edunol.

Compound G is white amorphous powder. $[\alpha]_D^{25} = -17.4°$. It has a molecular formula of $C_{21}H_{18}O_6$ as determined from the ion peaks at *m/z* 366 $[M]^+$ and 335$[M-CH_2OH]^+$ in the EI-MS and *m/z* 366.1092 $[M]^+$ in the HR-EI-MS. Its ^1H and ^{13}C NMR spectra (Table 1) were closely similar to those of emoroidocarpan (Palazzino *et al.*, 2003), except the absence of proton and carbon signals for 3′-Me. Instead, the spectra exhibited resonances indicating the presence of an oxygenated methylene [δ_H 4.28 (1H, d, J = 13.8 Hz), 4.24 (1H, d, J = 13.8 Hz); δ_C 63.0]. In the HMBC spectrum (Table 1), the oxygenated methylene protons were observed to be correlated with C-2′ (δ_C 84.6), C-3′ (δ_C147.4), and C-5′ (δ_C 112.3). These findings in combination with the molecular formula showed that a hydroxyl group is attached to C-4′ in this compound. The relative stereochemistry was deduced to be identical with that of emoroidocarpan from the ^1H NMR coupling constant (J = 6.8 Hz) between H-6a and H-11a and the NOESY spectrum (Table 1), in which an NOE interaction is observed between H-6a and H-11a. Thus, the compound is identified as 4′-hydroxyemoroidocarpan.

Table 1 ^1H and ^{13}C NMR data, and NOESY and HMBC correlations of 4′-hydroxyemoroidocarpan

Position	δ_H (J in Hz)	NOESY	δ_C	HMBC
1	7.27 s		126.5	C-3, 4a, 11a, 1′
1a			112.4	
2			120.6	
3			160.8	
4	6.41 s		98.5	C-1a, 2, 3, 4a
4a			156.1	
6	4.21 dd (5.0, 11.0), 3.62 t (11.0)	H-6a	66.6	
6a	3.47 ddd (5.0, 6.8, 11.0)	H_2-6, H-11a	40.2	
6b			118.0	
7	6.72 s		104.8	C-6a, 8, 9, 10a
8			141.7	
9			148.1	
10	6.43 s		93.8	C-6b, 8, 9, 10a
10a			154.2	
11a	5.47 d (6.8)	H-6a	79.0	C-1, 1a, 4a, 6, 6a
1′	3.36 dd (9.5, 15.5), 3.12 dd (8.0, 15.5)	H-2	34.4	C-1, 2, 3, 2′, 3′
2′	5.37 t (8.0)	H_2-1′	84.6	C-2, 3, 1′, 3′, 4′, 5′
3′			147.4	
4′	4.28 d (13.8), 4.24 (13.8)		63.0	C-2′, 3′, 5′
5′	5.27 br s		112.3	C-2′, 3′, 4′
OCH_2O	5.92 d (1.1), 5.89 d (1.1)		101.3	C-8, 9

Compound H is colourless needle crystal. mp: 150 – 152℃, $[\alpha]_D^{25} = -304°$ ($C_{0.05}$, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ_H 1.75 (3H, s, CH_3-OOC-), 3.65 (1H, t, J = 5.3 Hz, H-6), 3.85 (6H, s, 2 × OCH_3), 4.32 – 4.35 (1H, m, H-6), 4.53 (1H, t, J = 11.3 Hz, H-12a), 4.98 – 5.03 (1H, m, H-6a), 6.43 (1H, s, H-4), 6.44 (1H, d, J = 4.6 Hz, H-12), 6.69 (1H, s, H-1), 6.87 (1H, d, J = 2.0 Hz, H-3′), 7.11 (1H, d, J = 8.4 Hz, H-10), 7.21 (1H, d, J = 8.4 Hz, H-11), 7.57 (1H, d, J = 2.2 Hz, H-2′); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 112.0 (C-1), 143.6 (C-2), 146.9 (C-3), 100.2 (C-4), 148.7 (C-4a), 64.4 (C-6), 66.6 (C-6a), 149.5 (C-7a), 108.6 (C-8), 156.8 (C-9), 104.0 (C-10), 126.8 (C-11), 111.3 (C-11a), 69.1 (C-12), 36.7 (C-12a), 117.0 (C-12b), 144.3 (C-2′) 105.2 (C-3′), 56.5 (2-OMe), 55.9 (3-OMe), 20.8 (-CH_3), 170.4 (-COO-). The spectroscopic data is consistent with that listed in the literature (Lin *et al.*, 1993), so compound H is identified as 12-acetyelliptinol.

Compound I is brown crystal. ^1H NMR (500

MHz, $CDCl_3$): δ_H 3.78 (3 H, s, OCH_3), 5.97 (2 H, s, $-OCH_2O-$), 6.64 (1 H, s, H-3″), 6.83 (1 H, dd, J=2.1 Hz, 0.8 Hz, H-3′), 6.90 (1 H, s, H-6″), 7.50 (1 H, s, H-8), 7.68 (1 H, s, H-5), 7.69 (1 H, d, J=2.2 Hz, H-2′), 7.89 (1 H, s, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 56.8 (-OMe), 95.5 (C-3″), 99.5 (C-8), 101.5 ($-OCH_2O-$), 106.4 (C-3′), 110.3 (C-6″), 116.2 (C-1″), 116.2 (C-4a), 119.6 (C-5), 124.0 (C-3), 124.8 (C-6), 141.3 (C-5″), 142.4 (C-4), 146.7 (C-2′), 148.8 (C-4″), 151.6 (C-8a), 152.9 (C-2″), 156.1 (C-7), 160.7 (C-2). The spectroscopic data is consistent with that listed in the literature (Phrutivorapongkul *et al.*, 2002), so compound I is identified as pachyrrhizine.

Compound J is yellow crystal. mp: 101 – 103℃. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.63 (1H, d, J=9.5 Hz, H-4), 7.11 (1H, d, J=8.5 Hz, H-8), 6.90 (1H, d, J=8.5 Hz, H-7), 6.46 (1H, s, H-5), 6.24 (1H, d, J=9.5 Hz, H-3), 4.11 (3H, s, C_{11}-OMe); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 160.4 (C-2), 112.6 (C-3), 144.3 (C-4), 112.1 (C-5), 152.1 (C-6), 113.2 (C-7), 123.3 (C-8), 147.2 (C-9), 133.7 (C-10), 61.8 (C-11). The spectroscopic data is consistent with that listed in the literature (Kitamura *et al.*, 2003; Kotani *et al.*, 2004; Oyamada and Kitamura, 2006), so compound J is identified as 6-methoxycoumarin.

3.2 Insecticidal activity of compounds

The LC_{50} or LD_{50} values were as showed in Table 2. Based on the LC_{50} or LD_{50} values, ten potential insecticidal compounds against *A. albopictus* larvae were arranged in the following order from high to low: 12a-hydroxyrotenone > 4′-hydroxyemoroidocarpan > pachyrrhizine > rotenone > 6-methoxycoumarin > (-)-edunol > obovatin > pongachin > 12-acetyelliptinol > 2-hydroxyrotenone, with the LC_{50} values of 12.5, 22.1, 25.0 mg/L, 34.1, 43.4, 58.4, 121.9, 191.0, 219.8 and 250.0 mg/L, respectively. Three compounds, *i.e.*, 12a-hydroxyrotenone, rotenone and 4′-hydroxyemoroidocarpan, exhibited insecticidal activity against *M. persicae* adults and the 3rd larvae of *P. xylostella*, and their insecticidal potential against *M. persicae* adults was arranged in the following order from high to low: 12a-hydroxyrotenone > rotenone > 4′-hydroxyemoroidocarpan; and their insecticidal potential to the 3rd *P. xylostella* larvae was arranged in the following order from high to low: 12a-hydroxyrotenone > 4′-hydroxyemoroidocarpan > rotenone.

Table 2　Insecticidal activity of the compounds from *Tephrosia purpurea* bark against the 4th instar larvae of *Aedes albopictus*, the 3rd instar larvae of *Pluttella xylostella* and the adults of *Myzus persicae*

Compounds	Insects	Toxicity regression equation	LC_{50} (mg/L) (95% confidence interval)	LD_{50} (μg/individual) (95% confidence interval)	χ^2
4′-Hydroxyemoroidocarpan	*A. albopictus*	$y=2.3+1.9x$	22.1 (16.6 – 28.9)		0.8590
Rotenone		$y=2.9+1.4x$	34.1 (22.4 – 49.2)		0.5385
2-Hydroxyrotenone		$y=0.2+1.9x$	250.0 (190.3 – 333.5)		2.5078
12-Acetyelliptinol		$y=0.9+1.7x$	219.8 (163.1 – 302.1)		0.9553
12a-Hydroxyrotenone		$y=3.4+1.4x$	12.5 (8.3 – 17.7)		0.2567
Pachyrrhizine		$y=1.3+2.7x$	25.0 (18.9 – 30.5)		1.6413
Obovatin		$y=2.1+1.4x$	121.9 (83.1 – 179.0)		1.8289
6-Methoxycoumarin		$y=1.7+2.0x$	43.4 (32.5 – 56.5)		3.2719
(-)-Edunol		$y=2.8+1.2x$	58.4 (30.9 – 87.3)		0.2940
Pongachin		$y=0.2+2.1x$	191.0 (146.2 – 247.7)		0.8408
4′-Hydroxyemoroidocarpan	*P. xylostella*	$y=2.7+1.4x$		49.8 (33.7 – 73.4)	0.6642
Rotenone		$y=0.9+1.8x$		197.1 (144.4 – 265.2)	0.9732
12a-Hydroxyrotenone		$y=2.4+1.6x$		40.8 (28.2 – 55.8)	0.4494
4′-Hydroxyemoroidocarpan	*M. persicae*	$y=2.7+1.4x$	49.9 (33.7 – 73.4)		0.6642
Rotenone		$y=4.7+1.2x$	1.9 (1.1 – 2.8)		0.5257
12a-Hydroxyrotenone		$y=5.0+1.5x$	0.9 (0.6 – 1.4)		0.3984

4 DISCUSSION

In this study, ten known compounds with insecticidal property were isolated and identified from *T. purpurea* bark by activity-guided fractionation with column chromatography, including two coumarins (6-methoxycoumarin and pachyrrhizine) and eight flavonoids [rotenone, 12a-hydroxyrotenone, 4′-hydroxyemoroidocarpan, (-)-edunol, obovatin, pongachin, 12-acetyelliptinol, and 2-hydroxyrotenone]. Except rotenone and 12a-hydroxyrotenone, the others were isolated from *T. purpurea* bark for the first time.

Various compounds (including flavonoids, terpenoids, phenolics and alkaloids) existed in plant extracts and jointly or independently contributed to bioefficacy such as insecticidal, ovicidal, repellent, and antifeeding activities against various insect species (Isman, 2000). Some researchers focused on the determination of the distribution, nature, and practical use of plant extracts-derived chemical constituents with insecticidal activities (Pavela and Herda, 2007; Li *et al.*, 2007, 2011). The results of this study indicated that at least two classes of phytochemicals, flavonoids and coumarins, with insecticidal property existed in *T. purpurea* bark.

Flavonoids play a key role in stress response mechanisms in plants, which act as antioxidants or as enzyme inhibitors involved in photosynthesis and cellular energy transfer processes, and may serve as the precursor of toxic substances with insecticidal activity (Ververidis *et al.*, 2007). The adaptive role of flavonoids in plant defense against bacterial, fungal and viral diseases has been confirmed. The methanol extracts from *T. purpurea* bark exhibited insecticidal activity against various species of insect pests including *A. albopictus* larvae, with LC_{50} value being 97.7 mg/L against the 4th instar larvae of *A. albopictus* at 24 h after treatment (Li *et al.*, 2007). In this study, eight flavonoids and two coumarins independently exhibited insecticidal activity against the mosquito larvae (Table 2). Thus, insecticidal activity of the bark extracts against the mosquito larvae is due to the joint contribution of these compounds.

Earlier phytochemical research revealed that flavonoids including isoflavones, flavones, flavanones, chalcones, flavonols and rotenoids were the main constituents occurring in this plant (Sinha *et al.*, 1982). Within the group of flavonoids, 5, 7-oxygenated and 7-oxygenated compounds characterized by the presence of C-8 prenyl unit are well known. In many cases, these prenylated flavones have undergone further substitution and cyclization leading to complex molecules (Sinha *et al.*, 1982). Our experiment indicated that some prenylated flavonoids probably turn into more complex compounds because of the above-mentioned substitution and cyclization. Some flavonoids were isolated from *T. purpurea* bark for the first time, which provides proof to support the above-mentioned hypothesis.

Coumarins are also a major class of secondary metabolites in plants. To date there are about 900 coumarins documented in plants and the list is steadily increasing (Chen, 2004). Most of coumarins exhibit bioefficacy in plant defense against bacterial, fungal and viral diseases, and a few with light-sensitive character often exhibit insecticidal activities (Chen, 2004). In this study, two coumarins (6-methoxycoumarin and pachyrrhizine) exhibited insecticidal activity (Table 2), and their mechanism of action deserves to be further researched.

Elucidation of insecticidal compounds in plants is the basis to develop new botanical insecticides (Belmain *et al.*, 2001). *Derris elliptica*, *Azadirachta indica* and *Chryaanthemum cinerariaefotiumvis* are very successful examples. Azadirachtin isolated from *A. indica* showed great bioactivity against pests, so did rotenoids isolated from *D.* spp. and pyrethrin I isolated from *C. cinerariaefotiumvis* (Xu, 2001). Azadirachtin and rotenone have been explored and introduced into the market to control various species of agricultural pests, and pyrethrin I as a lead compound has been developed into a series of insecticides on the basis of structure optimization.

According to this study, 12a-hydroxyrotenone and 4′-hydroxyemoroidocarpan exhibited insecticidal activities against three species of pests (Table 2), especially to *P. xylostella* larvae, and the corresponding LD_{50} value is lower than that of the conventional botanic insecticidal compound rotenone, suggesting that the insecticidal activity of the two compounds against *P. xylostella* larvae is superior to that of rotenone. *P. xylostella* is one of the most important insect pests on brassica crops (Ma *et al.*, 2005). At present, chemical control of *P. xylostella* is becoming less effective because many populations of this pest developed resistance to some insecticides (Ma *et al.*, 2005). So the two compounds deserve to be further evaluated before they are developed into insecticides by structure optimization and directly used as insecticidal ingredient.

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References

Andrei CC, Ferreira DT, Faccione M, Faccione M, de Moraes LAB, de Carvalho MG, Braz-Filho R, 2000. C-prenylflavonoids from roots of *Tephrosia tunicata*. *Phytochemistry*, 55(7): 799 – 804.

Belmain SR, Neal GE, Ray DE, Golob P, 2001. Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food and Chemical Toxicology*, 39: 287 – 291.

Ben Jannet H, Harzalla-Skhiri F, Mighri Z, Simmonds MSJ, Blaney WM, 2000. Responses of *Spodoptera littoralis* larvae to Tunisian plant extracts and to neo-clerodane diterpenoids isolated from *Ajuga pseudoiva* leaves. *Fitoterapia*, 71: 105 – 112.

Charalambous A, Tluczek L, Frey KA, Higgins DSJr, Greenamyre TJ, Kilbourn MR, 1995. Synthesis and biological evaluation in mice of (2-[^{11}C] methoxy)-6′, 7′-dihydrorotenol, a second generation rotenoid for marking mitochondrial complex I activity. *Nuclear Medicine and Biology*, 22: 491 – 496.

Chen YG, 2004. Phytochemistry. Chemical Industry Press, Beijing. [陈业高, 2004. 植物化学成分. 北京: 化学工业出版社]

Huang GY, 2000. Pesticides Experiment Technology and Evaluation Methods. China Agriculture Press, Beijing. 10 – 19. [黄国洋, 2000. 农药试验技术与评价方法. 北京: 中国农业出版社. 10 – 19]

Huang SQ, Zhang ZX, Li YZ, Li YX, Xu HH, 2010. Anti-insect activity of methanol extracts of fern and gymonosperm. *Agricultural Sciences in China*, 9: 249 – 256.

Isman MB, 2000. Plant essential oils for pest and disease management. *Crop Protection*, 19: 603 – 608.

Khan N, Sharama S, Alam A, Saleem M, Sultana S, 2001. *Tephrosia purpurea* ameliorate N-diethylnitrosamine and potassium bromate-mediated renal oxidative stress and toxicity in Wistar rats. *Pharmacology and Toxicology*, 88: 294 – 299.

Kitamura T, Yamamoto K, Kotani M, Oyamada J, Jia CG, Fujiwara Y, 2003. Pd^{II}-catalyzed reaction of phenols with propiolic esters. A single-step synthesis of coumarins. *Bulletin of the Chemical Society of Japan*, 76(10): 1889 – 1895.

Kotani M, Yamamoto K, Oyamada J, Fujiwara Y, Kitamura T, 2004. A convenient synthesis of coumarins by palladium (II)-catalyzed reaction of phenols with propiolic acids. *Synthesis*, (9): 1466 – 1470.

Li GH, Wang SN, Zeng DQ, Yao ZW, Li YZ, 2011. The toxic action of essential oils from *Tephrosia purpurea* on *Rhizopertha dominica*. *Journal of Hunan Agricultural University* (*Natural Science*), 37(2): 181 – 184. [李冠华, 王苏宁, 曾东强, 姚振威, 李有志, 2011. 灰毛豆精油对谷蠹的毒杀作用. 湖南农业大学(自然科学版), 37(2): 181 – 184]

Li YZ, Huang SQ, Xu HH, 2007. Insecticidal activity of methanol extracts from *Tephrosia purpurea*. *Chinese Bulletin of Entomology*, 44: 680 – 685. [李有志, 黄素青, 徐汉虹, 2007. 灰毛豆甲醇提取物的杀虫活性. 昆虫知识, 44: 680 – 685]

Li YZ, Xu HH, 2007. Insecticidal activity and active compounds of *Derris cavaleriei*. *Scientia Agricultura Sinica*, 40(8): 1688 – 1696. [李有志, 徐汉虹, 2007. 湘西黑藤的杀虫活性及其杀虫成分. 中国农业科学, 40(8): 1688 – 1696]

Lin YL, Chen YL, Kuo YH, 1993. A novel 12-deoxorotenone, 12-deoxo-12-acetoxyelliptone, from the roots of *Derris oblonga*. *Journal of Natural Products*, 56: 1187 – 1189.

Ma J, Li YZ, Keller M, Ren SX, 2005. Functional response and predation of *Nabis kinbergii* (Hemiptera: Nabidae) to *Plutella xylostella* (Lepidoptera: Plutellidae). *Insect Science*, 12: 155 – 162

Nathan SS, Choi MY, Paik CH, Seo HY, 2007. Food consumption, utilization, and detoxification enzyme activity of the rice leaffolder larvae after treatment with *Dysoxylum* triterpenes. *Pesticide Biochemistry and Physiology*, 88: 260 – 267.

Oyamada J, Kitamura T, 2006. Synthesis of coumarins by Pt-catalyzed hydroarylation of propiolic acids with phenols. *Tetrahedron*, 62(29): 6918 – 6925.

Palazzino G, Rasoanaivo P, Federici E, Nicoletti M, Galeffi C, 2003. Prenylated isoflavonoids from *Millettia pervilleana*. *Phytochemistry*, 63: 471 – 474.

Pavela R, Herda G, 2007. Repellent effects of pongam oil on settlement and oviposition of the common greenhouse whitefly *Trialeurodes vaporariorum* on chrysanthemum. *Insect Science*, 14: 219 – 224.

Phrutivorapongkul A, Lipipun V, Ruangrungsi N, Watanabe T, Ishikawa T, 2002. Studies on the constituents of seeds of *Pachyrrhizus erosus* and their anti herpes simplex virus (HSV) activities. *Chemical and Pharmaceutical Bulletin*, 50(4): 534 – 537.

Rao EV, Raju NR, 1984. Two flavonoids from *Tephrosia purpurea*. *Phytochemistry*, 23(10): 2339 – 2342.

Reyes-Chilpa R, Gómez-Garibay F, Quijano L, Maagos-Guerrero GA, Ríos T, 1994. Preliminary results on the protective effect of (-)-edunol, a pterocarpan from *Brongniartia podalyrioides* (Leguminosae), against *Bothrops atrox* venom in mice. *Journal of Ethnopharmacology*, 42: 199 – 203.

Sinha B, Natu AA, Nanavati DD, 1982. Prenylated flavonoids from *Tephrosia purpurea* seeds. *Phytochemistry*, 21: 1468 – 1470.

Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N, 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part II: reconstruction of multienzyme pathways in plants and microbes. *Biotechnol. J.*, 2: 1235 – 1249.

Waterman PG, Mahmoud EN, 1985. Flavonoids from the seeds of *Lonchocarpus costaricensis*. *Phytochemistry*, 24(3): 571 – 574.

Xu HH, 2001. Insecticidal Plants and Botanical Insecticides. China Agriculture Press, Beijing. [徐汉虹, 2001. 杀虫植物与植物性杀虫剂. 北京: 中国农业出版社]

灰毛豆树皮中的杀虫成分及其杀虫活性

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摘要：为确定灰毛豆 *Tephrosia purpurea* 树皮甲醇提取物中的杀虫成分，以白纹伊蚊 *Aedes albopictus* 4 龄幼虫为靶标昆虫，在活性跟踪的基础上利用色谱技术分离其活性成分，然后根据各化合物的核磁共振图谱和质谱数据确定化合物的结构，并利用玻片载蚜法和点滴法测定了各化合物对桃蚜 *Myzus persicae* 无翅蚜成虫和小菜蛾 *Pluttella xylostella* 3 龄幼虫的毒杀活性。结果表明：从该植物树皮甲醇提取物中共分离、鉴定了 10 个对白纹伊蚊幼虫具有毒杀作用的化合物，即 12a-羟基鱼藤酮（12a-hydroxyrotenone），4′-hydroxyemoroidocarpan，豆薯内酯（pachyrrhizine），鱼藤酮（rotenone），6-甲氧基香豆素（6-methoxycoumarin），(-)-edunol，obovatin，pongachin，12-acetyelliptinol 和 2-hydroxyrotenone。这些化合物对该蚊虫幼虫处理 24 h 的 LC_{50} 值分别是 12.5，22.1，25.0，34.1，43.4，58.4，121.9，191.0，219.8 和 250.0 mg/L。3 个化合物（4′-hydroxyemoroidocarpan，鱼藤酮和 12a-羟基鱼藤酮）对桃蚜成虫和小菜蛾 3 龄幼虫表现出毒杀活性，它们对桃蚜 24 h 的 LC_{50} 值分别是 49.9，1.9 和 0.9 mg/L，对小菜蛾幼虫 24 h 的 LD_{50} 值分别是 49.8，197.1 和 40.9 μg/头。首次从该植物中分离得到 6 个已知的黄酮类化合物［4′-hydroxyemoroidocarpan，2-hydroxyrotenone，(-)-edunol，12-acetyelliptinol，pongachin 和 obovatin］和 2 个已知的香豆素类化合物（6-甲氧基香豆素和豆薯内酯）。阐明这些杀虫化合物的结构不仅有利于理解植物和昆虫的关系，而且有助于评价该植物及其活性化合物作为植物源农药开发利用的潜力。

关键词：灰毛豆；杀虫化合物；杀虫活性；白纹伊蚊；小菜蛾；桃蚜

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